knee compared to those without such signs. While replication is pursued, these findings establish CLEC4A as a potential biomarker for early knee OA.

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HERITABILITY ASSESSMENT OF CARTILAGE METABOLISM. A TWIN STUDY ON PROCOLLAGEN II N-TERMINAL PROPEPTIDE (PIIANP)

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Purpose: The aim of the study was to estimate heritability on circulating collagen
IIA N-propeptide (PIIANP) by studying mono- and dizygotic healthy twin pairs.
PIIANP: Collagen is produced and secreted as a precursor molecule with C-and N-terminal extensions termed procollagen peptides. In the extracellular space these are cleaved off by specific C- and N-peptidases after which collagen can participate in fibril formation. Since procollagen propeptides are released from the parent molecule in a stoichiometric manner, the concentration of these peptides provides an opportunity to assess the current biosynthetic activity.
Methods: A total of 602 healthy monozygotic (MZ) and dizygotic (DZ) twin individuals aged 18-55 years were recruited from the Danish Twin Registry. PIIANP was measured by competitive ELISA.
The similarity of circulating PIIANP among MZ and DZ twins was assessed by means of intraclass correlations for the traits. Classic twin study methodology is based on the fact that MZ twins have identical segregating genotypes, whereas DZ twins share, on average, one-half of their DNA sequence variation just like ordinary siblings. A greater phenotypic similarity in MZ than in DZ twins is anticipated if there is a significant genetic influence on the trait studied.
I agreement with standard practice, we assume no epistasis (genetic inter-locus interaction) and no gene-environment interaction or correlation. The extent to which variation in a trait is attributable to genetics (heritability) can be estimated quantitatively through variance components analysis. The phenotypic (P) variance in a trait can be separated into four variance components: variance due to additive genetic effects (A), genetic dominance (D), shared (family) environment (C) and non-shared(individual) environment (E), e.i., P = A + D + C + E. It is not possible to test all 4 components of this model at the same time; we therefore tested different models in a stepwise deletion process.
Results: The correlation of the logarithmic values of serum PIIANP for the MZ and the DZ twins is presented in figure 1.

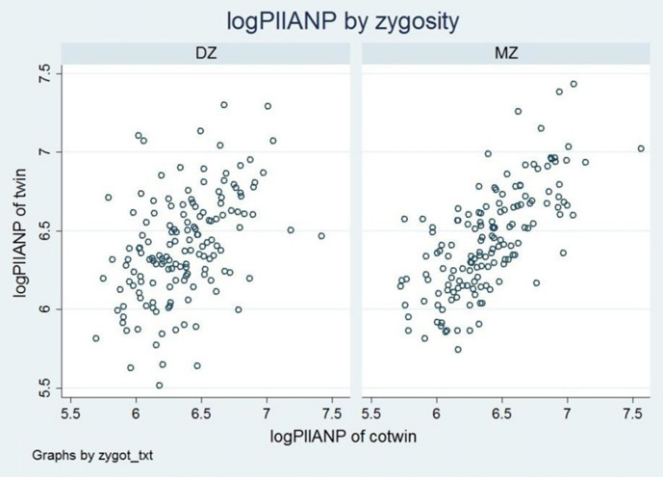

Figure 1. Correlation diagrams for log(PIIANP) in DZ and in MZ twins. Each dot represents the logarithm of the serum PIIANP level in a pair of twins, with one twin assigned to the abscissa and the other to the ordinate.

The intraclass correlation for PIIANP in MZ twins and DZ twins was 0.70(S.E. 0,042) vs. 0.45(S.E. 0,065). This indicates that genetic factors are of aetiological importance for the serum level of PIIANP. In the variance component analysis the most suitable model was an ACE model, where fortyfive percent of the phenotypic variance of PIIANP in serum is determined by genes, while the shared environment accounts for 24% and 31% can be explained by non-shared environment factors. Our study also shows, that among all the tested variables, sex was the only variable which was significantly associated with the PIIANP level in serum.
Conclusions: Our findings suggest that genetic factors have a considerable impact on the serum level of PIIANP. However, shared and common environmental factors are important modifiers as well.

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PROTEOMIC ANALYSIS OF CONNEXIN 43 REVEALS NOVEL INTERACTORS, NUCLEAR TRANSLOCATION AND ASSOCIATION WITH PROTEINS DYSFUNCTIONAL RELATED WITH OSTEOARTHROSIS

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Purpose: Human adult articular cartilage is composed of a dense extracellular matrix and specialized cells called chondrocytes. The chondrocytes are found to their own lacuna and remain in resting stage refraining form proliferation but displaying a moderate metabolic activity to maintain their surrounding matrix during the whole adult life. We have previously found that human adult chondrocytes express the gap junction (GJ) protein connexin 43 and chondrocytes in tissue have long cytoplasmic arms that physically connect two distant cells. GJ channels achieve direct cellular communication by allowing the intercellular exchange of ions, several molecules and second messengers. In addition, several GJ channel-independent functions have been related with Cx43. The interaction of proteins with the C-terminal tail of Cx43 directly modulates different cellular activities such as cell growth and proliferation.
Methods: In situ cartilage was immediately frozen after surgery in a Cryomold® Standard using Tissue-Tek® O.C.TTM Compound and stored at -80°C to keep the pattern of expression and protein levels. Chondrocytes were isolated by sequential digestion with trypsin-EDTA and Collagenase. Isolated cells from healthy and cartilage from osteoarthritis (OA) patients were maintained for stable short-term cell culture in DMEM supplemented with primocin and 15% FCS. For immunohistochemistry (IHC) experiments cells were seeded on chamber slides and fixed with acetone. Co-immunoprecipitation (IP) experiments were performed to identify the proteins that interact with the C-Terminal tail of Cx43. In-gel digestion of immunoprecipitated proteins separated by SDS-PAGE were analysed using the nano-liquid chromatography (Nano-LC, Eksigent) coupled to mass spectrometry (MALDI-TOF/TOF, Applied Biosystem). The identification of proteins was performed using ProteinPilot™ 3.0 Software. Samples were evaluated by SDS-PAGE followed by Western blotting with specific antibodies.
Results: A total of 200 were identified, of which 131 proteins were represented by at least two unique peptides. 103 proteins were specific to the Cx43 IP, not identified in the control IP performed without antibody. Identified interactors show significant enrichment for Gene Ontology (GO) processes directly linked with cytoskeleton dynamics, metabolic pathways, nuclear activity and translation such as ß-tubulin, vimentin, GAPDH, histone H3 and H4, and several ribosomal-related proteins. IHC experiments showed that chondrocytes from OA patients in cartilage contain higher levels of Cx43 in the nucleus, the cytoplasm and membrane. However Cx43 was only found in the membrane of healthy chondrocytes in tissue.
Conclusions: Lysates of primary articular chondrocytes were subjected to pull-down assays and the identification of proteins by MALDI-TOF/TOF identify >100 Cx43 associated proteins. Mass-spectrometry results revealed novel functional Cx43 interactors involved in human disease and OA development emphasizing the importance of Cx43-interactions for normal development and physiology. Besides IHC experiments suggest that Cx43 interacts with nuclear and translational components especially in OA cartilage.

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A NOVEL OA EFFICACY MARKER: CARTILAGE ACTIVITY

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Purpose: The pathogenesis of OA is complex with multiple events occurring simultaneously in the joint. The opposing cartilage effects of